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ON THE NATURE OF THE OSCILLATIONS OF THE MEMBRANE POTENTIAL (SLOW WAVES) PRODUCED BY ACETYLCHOLINE OR CARBACHOL IN INTESTINAL SMOOTH MUSCLE

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SUMMARY

1. Intracellular recording was made with glass micro-electrodes from cells of the longitudinal muscle of the guinea-pig ileum in isotonic and in hypertonic solution.

2. In isotonic solution spontaneous bursts of electrical activity occurred; these consisted of a slow potential component which carried a burst of spike action potentials. Acetylcholine increased the size (and the frequency) of the slow potential component. This had the effect of first reducing and then abolishing the spike potentials; continuous slow wave activity was thus produced. Slow waves were about 1 sec in duration and up to 50 mV in size in isotonic solution.

3. In hypertonic solution the membrane potential was stable. There were no spontaneous spikes and no slow potentials. However, spikes, but not slow potentials, were elicited by depolarizing current. Carbachol (or acetylcholine) reduced the membrane potential and initiated spikes and oscillations of the membrane potential (slow waves). Slow waves were 2–5 sec in duration and 10–40 mV in size in hypertonic solution.

4. The response to carbachol in hypertonic solution was unaffected by surgical denervation of the tissue, by tetrodotoxin, or by ganglion blocking agents, indicating that muscarinic stimulants produced their effects by acting directly on the smooth muscle cell.

5. In hypertonic solution slow waves occurred only in the presence of a muscarinic stimulant and could not be elicited with depolarizing current (unless carbachol was present) nor by increasing the external potassium concentration.

6. In hypertonic solution slow waves were abolished by hyperpolarizing the membrane and their rate of rise was proportional to the level of the membrane potential from which they arose. The membrane resistance was

reduced at the peak of the slow wave. Slow waves were rapidly abolished by sodium-deficient solutions but spikes were not.

7. It is suggested that slow waves represent an inward current through a slow, sodium-sensitive and voltage-dependent ion channel, and that acetylcholine or carbachol increase, while hypertonic solution decreases, the current carried by this channel.

INTRODUCTION

In most excitable membranes the action of transmitters can be explained by assuming that activation of the receptor opens additional ion channels in the membrane. The opening of these extra channels causes the membrane potential to move towards the equilibrium potential for the transmitter. At the end-plate of skeletal muscle fibres (Fatt & Katz, 1951; Takeuchi & Takeuchi, 1959, 1960) and in almost all other situations that have been studied (Ginsborg, 1967), the conductance change produced by the transmitter is independent of the level of the membrane potential. This has the effect that the action of an excitatory transmitter is, to a first approximation, rather similar to depolarizing the membrane. In fact, careful study will usually reveal the effects of the additional conductance opened by the transmitter on the resulting electrical activity (e.g. Fatt & Katz, 1951; Maeno, 1966).

It is known that acetylcholine depolarizes the membrane of smooth muscle of taenia coli (Bülbring, 1954, 1955; Bülbring & Kuriyama, 1963) and that it increases spike frequency and membrane conductance (Hidaka & Kuriyama, 1969; Kuriyama, 1970). Ion flux studies on smooth muscle indicate that acetylcholine or carbachol may increase the permeability to K^+, Cl^-, Br^-, Na^+ and Ca^{2+} (Born & Bülbring, 1956; Durbin & Jenkinson, 1961; Jenkinson & Morton, 1967; Burgen & Spero, 1968). However, the above observations do not explain the oscillations which acetylcholine produces in the longitudinal ileal muscle of the guinea-pig (Bolton, 1971).

METHODS

Guinea-pigs of 200–400 g body weight were killed and the terminal 15 cm of the ileum proximal to the ileo-caecal lymph node were removed and a glass rod inserted into the lumen. A longitudinal strip about 2 or 3 mm wide, consisting of the whole thickness of the ileal wall was cut. A few experiments were done on longitudinal muscle which was separated from the underlying circular muscle by a method similar to that described by Paton & Zar (1968). The longitudinal strip of tissue was introduced into apparatus described by Abe & Tomita (1968). This apparatus enables current to be passed through the tissue by means of extracellular electrodes while intracellular recording is made in the extrapolar region. Silver–silver chloride recording electrodes situated in the mid-interpolar region record the size of the voltage gradient applied to the tissue and this is displayed as a deflexion of the upper trace

in the illustrations. The current through the membrane is assumed to be proportional to the applied voltage. The membrane potential changes of smooth muscle cells of the longitudinal layer were recorded with potassium chloride-filled glass microelectrodes of 40–100 MΩ resistance.

The isotonic solution used the following composition (mM): NaCl 120, KCl 5·9, $CaCl_2$ 2·5, $MgCl_2$ 1·2, NaH_2PO_4 1·2, $NaHCO_3$ 15, glucose 11. Hypertonic solution had the following composition (mM): NaCl 110, KCl 5·4, $CaCl_2$ 2·3, $MgCl_2$ 1·1, NaH_2PO_4 1·1, $NaHCO_3$ 14, glucose 10, sucrose 410. Sodium-deficient hypertonic solution was made by replacing sodium chloride with Tris titrated to pH 7·4 with hydrochloric acid. Solutions were equilibrated with 5% CO_2 and 95% CO_2 before use. Acetylcholine or carbachol (concentrations expressed as the chloride salt) was applied to the tissue by changing the bathing solution for another of identical composition but containing the drug.

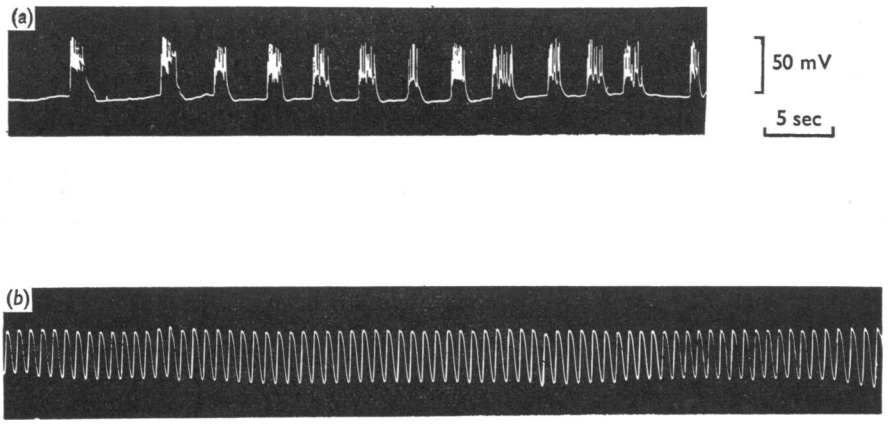

Fig. 1. Action of acetylcholine in isotonic solution at 35° C. (*a*) Spontaneous membrane activity. This consists of periodical slow potential changes which carry a burst of action potentials. (*b*) Slow wave activity evoked by acetylcholine (10^{-8} g/ml).

RESULTS

The response to acetylcholine or carbachol in isotonic and hypertonic solutions

Isotonic solution. In isotonic solution the membrane was spontaneously active. Kuriyama, Osa & Toida (1967*b*) have described the bursts of activity as consisting of a slow potential change topped by bursts of spikes (Fig. 1*a*). Acetylcholine (10^{-8} g/ml.) converted this type of activity to oscillations of the membrane potential, or slow waves, which had a period of oscillation of about 1 sec and which could be over 50 mV in size (Fig. 1*b*). The transition from spontaneous to slow wave activity is illustrated in Fig. 2. Acetylcholine (10^{-8} g/ml.) enhanced the slow potential component, thus reducing the size of the spikes (Fig. 2*a–c*). Vestiges of spikes were often seen on the crests of the slow waves (Fig. 2*e*). At the peak of its

action, acetylcholine reduced the membrane potential (Fig. 2*c*) but depolarization was not the sole cause of the change in membrane activity because slow waves continued when the membrane potential had returned to, or even beyond, its resting level (Fig. 2*d–e*).

Hypertonic solution. In hypertonic solution the membrane potential was usually stable and lay between 50 and 60 mV if the deflexion of the trace upon presumed penetration of a cell can be taken as an indication of this parameter. Occasional preparations discharged action potentials continuously and regularly. It is likely that hypertonic solution has a direct stabilizing effect on the membrane (cf. Tomita, 1966) possibly because it reduces its permeability to ions.

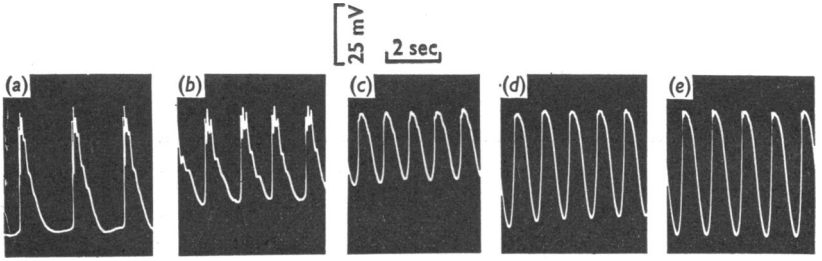

Fig. 2. Changes in membrane activity brought about by acetylcholine in isotonic solution. Acetylcholine enhances the slow potential component, thus reducing the size of the spikes (*a*, *b*). Slow waves result when this process is carried to the limit (*c*, *d*, *e*). The changes are not dependent upon the level of the membrane potential (cf. (*a*) and (*b*); (*c*), (*d*), and (*e*)).

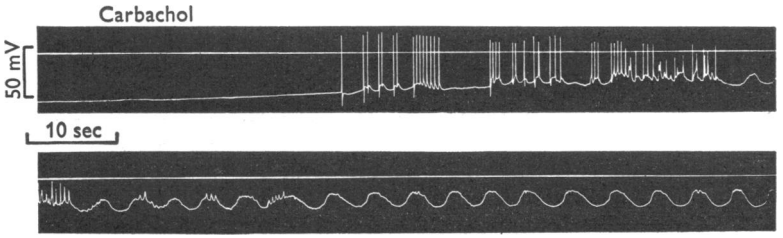

Fig. 3. Action of carbachol (5×10^{-7} g/ml.) on the membrane potential (lower line) in hypertonic solution at 35° C, showing that spikes and slow waves are separate phenomena. A few seconds of the trace are missing between the upper and lower records. The upper is a reference line in this and subsequent Figures.

Acetylcholine and carbachol were of similar potency and produced indistinguishable responses. The response to carbachol (2 to 5×10^{-7} g/ml.) showed little variation and is illustrated in Fig. 3. The first phase of the response consisted of a slow reduction in the membrane potential. This reduction generally amounted to less than 5 mV before the first action

potential occurred. A number of action potentials were then discharged; these were generally arranged in groups of two or more, each group associated with a slow wave of depolarization. This activity is strikingly similar to the spontaneous activity observed in isotonic solution. In some instances, one or more action potentials were fired singly before slow waves appeared. Single action potentials, and the first action potential of a group, often showed a marked after-hyperpolarization which often exceeded the size of any subsequent slow wave. After a variable period during which the action potentials declined slightly in size, only the slow waves remained, sometimes with vestiges of action potentials superimposed upon them. Slow waves were between 10 and 40 mV in size and had a surprisingly constant period of oscillation in any experiment, which was between 2 and 5 sec. They persisted for more than 20 min if carbachol or acetylcholine was present in the bathing solution. At the peak of the response the waves were almost sinusoidal. As the action of the drug declined, and the membrane repolarized, the rising phase of the slow wave became progressively steeper and eventually action potentials came to precede each wave. The response to acetylcholine or carbachol in hypertonic solution suggested that two distinct mechanisms may operate in this smooth muscle cell membrane, one of these mechanisms responsible for the action potential and the other for the slow wave.

Since, in hypertonic solution, spikes and slow waves were clearly separable phenomena, the experiments which will now be described were performed in hypertonic solution.

Evidence that carbachol is acting directly on the smooth muscle cell

As the preparation used consisted of the whole thickness of ileal wall, it seemed possible that carbachol or acetylcholine might induce a rhythmic and synchronous discharge in nerves which was reflected in slow wave activity of the smooth muscle membrane. Surgically denervated preparations (Paton & Zar, 1968) were therefore prepared and it was found that in all cells where the response to carbachol could be followed, there was marked slow wave activity in response to carbachol exactly as in preparations consisting of the whole thickness of the ileal wall. However, it may be argued that it is impossible to surgically eliminate extremely fine nerve terminals and that slow wave activity may still result from the action of carbachol (or acetylcholine) on these in a manner analogous to that suggested by Koelle (1962) in ganglia and by Riker, Werner, Roberts & Kuperman (1959) at the neuromuscular junction.

Further experiments were therefore done using pharmacological agents. Ganglion blockers, hexamethonium (2×10^{-5} g/ml.) and pentolinium (5×10^{-6} g/ml.) and also tetrodotoxin (up to 5×10^{-7} g/ml.) alone, or in

combination with surgical denervation, were without effect on slow waves produced by carbachol. All effects of carbachol or acetylcholine were of course completely abolished by hyoscine or atropine (10^{-8} g/ml.). These results strongly suggest that slow wave activity is brought about as the result of the interaction of the muscarinic agonist with the receptors on the smooth muscle cell and not indirectly.

However, neither ganglion blocking agents nor tetrodotoxin abolish the spontaneous release of acetylcholine from nerve terminals (Elmqvist & Feldman, 1965; Miledi, 1967; Landau, 1969), thus the theoretical possibility exists that a muscarinic agonist may still induce a cyclical release of acetylcholine from these. It is difficult to accept, however, when all other nerve activity is presumably abolished by tetrodotoxin or ganglion blocking agent, that nerve terminals can cyclically release sufficient acetylcholine to superimpose a slow wave pattern of activity of the magnitude shown in Figs. 1–3, on a membrane potential presumably already reduced by a constant concentration of the same substance added to the bathing fluid.

It may be argued that slow waves are a phenomenon which is dependent upon the geometry of the tissue, a slow oscillation of the membrane potential of a single cell resulting from the spatial and temporal summation of activity in adjacent fibres. This is a difficult proposition to disprove. However, the size and the period of oscillation of slow waves was not dependent on the geometry of the tissue: slow waves were not noticeably different whether recorded from cells in a small strip of separated (and rather traumatized) longitudinal muscle, from cells in a strip consisting of the whole thickness of ileal wall, or from cells in a segment of whole ileum several centimetres long. Furthermore, slow waves were always observed in response to adequate concentrations of carbachol. It is reasonable to conclude that when slow waves are observed in any cell, they are fundamentally a reflexion of a change in the activities (e.g. ion pumping) or properties (e.g. ion permeability) of that cell or its membrane. This leaves unanswered the effects of electrotonic interaction between cells on the observed responses.

The bursts of spontaneous activity which were observed in isotonic solution were unaffected by hyoscine in concentrations (10^{-6}–10^{-7} g/ml.) which might be expected to seriously impair any activity which was dependent upon the activation of a muscarinic receptor. These slow potentials and associated action potentials are therefore myogenic. Nevertheless, there is an appreciable release of acetylcholine, probably from nervous elements, in this tissue (Feldberg & Lin, 1949; Paton & Zar, 1968) and it is not inconceivable that on some occasions spontaneously released acetylcholine may produce additional slow wave activity which, of course, would be blocked by hyoscine or atropine (Kuriyama, Osa & Toida, 1967*c*).

Dependence of slow waves on the presence of a muscarinic stimulant

It seemed possible that slow waves may not be peculiar to the action of muscarinic stimulants, but might be a voltage-dependent property of the smooth muscle cell membrane which is manifested when the membrane

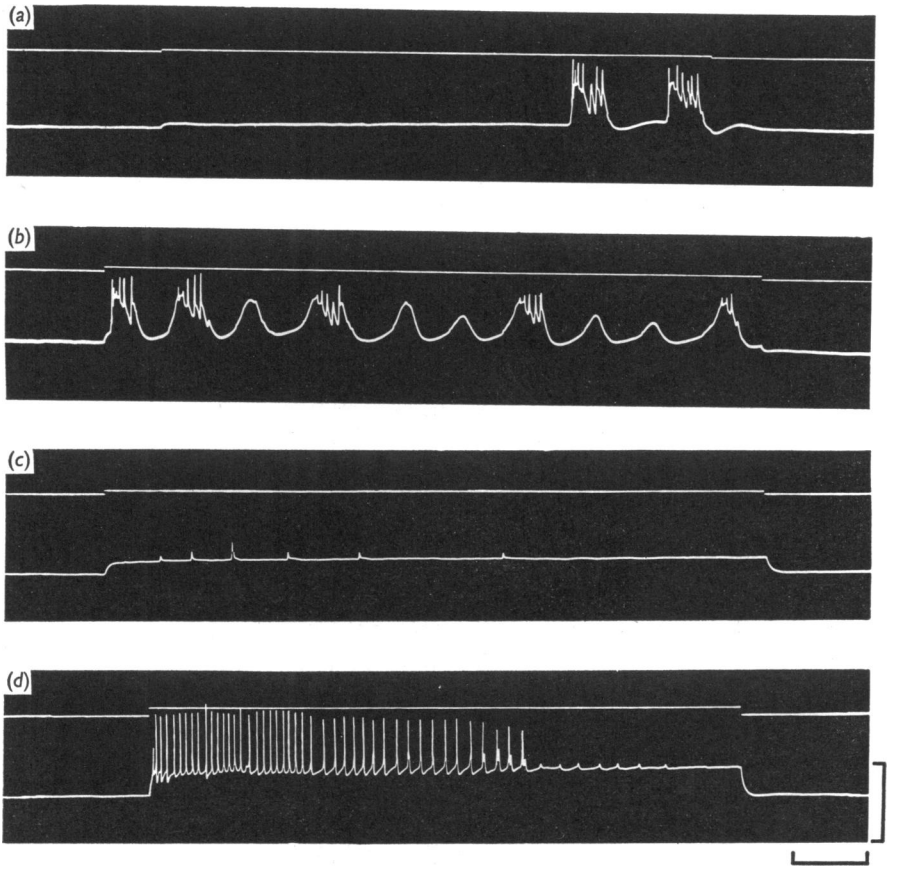

Fig. 4. Effect of a subthreshold concentration of carbachol on the response to depolarizing current. All records from the same cell in hypertonic solution, 35° C. Records (*a*) and (*b*) show the effect of outward current applied in the presence of a constant concentration of carbachol. Between (*b*) and (*c*) the tissue was returned to a carbachol-free solution and its effects allowed to decline. Records (*c*), (*d*), constant outward currents applied in the absence of carbachol. Notice that there is no evidence of slow waves in the absence of carbachol although the depolarizations produced are very similar and a vigorous spike discharge occurs. The deflexion of the upper trace is proportional to the voltage gradient applied to the tissue. (*a*) and (*c*) show the effect of subthreshold currents and (*b*) and (*d*) the effect of suprathreshold currents. The vertical calibration is 50 mV and the horizontal calibration 5 sec.

potential is reduced below its resting level. This possibility was tested by passing outward current. If the reduction in membrane potential was greater than a few millivolts, action potentials were discharged. The rate of firing decline during prolonged current application and spike discharge often ceased (Fig. 4*d*). There was no evidence, however, that action potentials were discharged in groups, or that slow waves or slow potentials occurred. This was true even if prolonged periods of current application were used, similar in duration to a depolarization produced by acetylcholine or carbachol.

However, if carbachol (or acetylcholine) was applied to the same cells, characteristic slow wave activity was elicited. This is shown in Fig. 4, which consists of records from a single cell. A concentration of carbachol was first introduced to the bathing solution which was sufficient to depolarize the cell by several millivolts, but was not sufficient by itself to elicit action potentials or slow waves. The cell was then further depolarized by passing outward current. If the depolarization was sufficiently large, marked slow wave activity appeared with some spikes (Fig. 4*b*). Carbachol was then removed from the bathing solution and its effects allowed to decline. Outward current was again passed. In this same cell only action potentials were discharged and no slow waves were seen (Fig. 4*d*). It is clear that the effects of outward current in the presence and in the absence of carbachol are very different.

When the membrane was depolarized by other means, such as high potassium (29·5–72 mM) or by histamine, no slow wave activity was seen. This result was somewhat surprising in view of the fact that in the longitudinal muscle of the guinea-pig ileum, high external potassium is considered to act in part by releasing acetylcholine from nerves (e.g. Paton & Zar, 1968). Histamine in hypertonic solution was very inactive but it produced occasional sporadic bursts of action potentials.

Changes in membrane resistance during the slow wave

The size of a transmembrane potential change recorded by a microelectrode inserted at a distance x from the point of injection of a current into a cable-like cell is approximately proportional to the square root of the membrane resistance when x is close to zero (Fatt & Katz, 1951, p. 334).

When electrotonic potentials were induced with rectangular pulses during slow wave activity, they were observed to vary in size, there being a reduction in their size at the peak of the slow wave (Fig. 5*a* and Fig. 7, lower line, middle panels). When the square of the size of electrotonic potentials was plotted, the membrane resistance and membrane potential were found to vary in parallel (Fig. 5*b*). In contrast, if the membrane was depolarized with constant outward current to a more positive potential

than the peak of the slow waves, then these were abolished. However, despite the marked depolarization, the membrane resistance was barely less than the resistance at the troughs of the slow waves, and greater than the resistance attained at their peaks. These observations suggest that some permeability change underlies the slow wave.

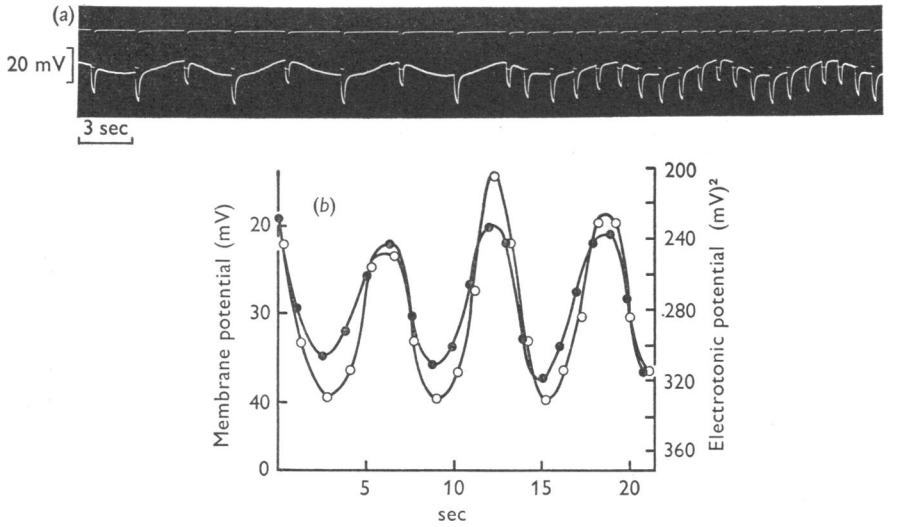

Fig. 5. The change in membrane resistance during the slow wave (*a*). Brief rectangular hyperpolarizing pulses were applied as indicated during slow wave activity induced by carbachol in hypertonic solution. In (*b*) the membrane potential (●) and membrane resistance (○), are plotted against time. The data comes from the last three waves in (*a*). The membrane resistance was expressed as the square of the size of the electrotonic potential.

Voltage dependence of slow waves

The experiment illustrated in Fig. 4 shows that the mechanism responsible for carbachol-induced slow waves was voltage dependent. A number of other observations supported this. If the membrane was hyperpolarized by passing current during slow wave activity, then slow waves were abolished. Similarly, if the membrane was depolarized with current beyond about -20 mV, slow waves were also abolished. A brief depolarizing pulse, applied as the effects of carbachol waned, when the membrane potential was again stable, triggered a slow wave of much longer duration than the applied pulse. When a concentration of carbachol sufficient to depolarize the membrane to a steady potential of about -15 mV was applied, slow waves were abolished; if the membrane was repolarized during a carbachol depolarization by passing current, then large slow waves occurred.

The rate of rise of slow waves was proportional to the level of the mem-

brane potential from which they arose. When the membrane potential was varied by passing constant current (Fig. 4) it was found that the rate of rise of slow waves was linearly related to the potential from which they arose. This is illustrated in Fig. 6*a*; there was some scatter of the points about the calculated regression line but the slope of the line is very significantly ($P < 0{\cdot}001$) different from zero. If action potentials were discharged from slow waves, this relationship was obscured. Conversely, the duration of the slow waves was not dependent upon the level of the membrane potential from which they arose (Fig. 6*b*). In this case, the slope of the regression line was not significantly ($P > 0{\cdot}5$) different from zero.

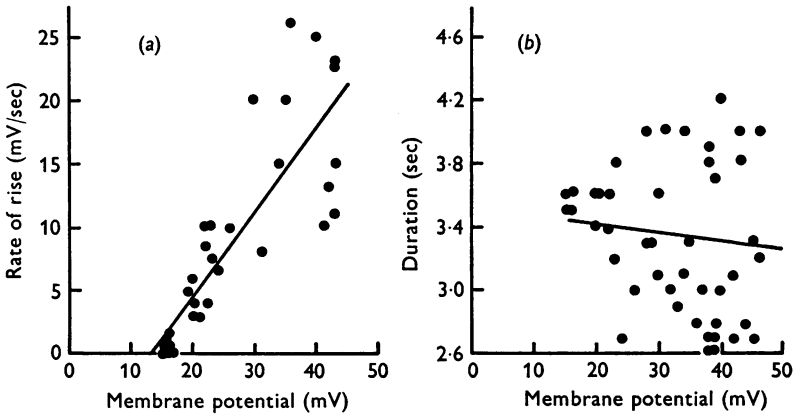

Fig. 6. The effect of the level of the membrane potential on the rate of rise of the slow wave (*a*) and its duration (*b*). The data are from the same experiment as Fig. 4. The position of the membrane potential from which the slow waves arose was adjusted by passing current. The calculated regression lines are shown. The slope in (*a*) is significantly ($P < 0{\cdot}001$) different from zero.

Equivalence of slow wave, slow potential and negative after-potential

Many records suggested that slow waves were in fact generated by the same mechanism as the negative after-potential which appeared as part of the action potential in the presence of carbachol. This was seen most clearly when the temperature was lowered because, under these circumstances, the number of action potentials was reduced. An experiment performed at 27° C is shown in Fig. 7. The first part of the record shows the onset of the action of carbachol. The first action potential was of a simple spike shape but successive action potentials were followed closely by a longer plateau of depolarization which increased in size as the effects of carbachol increased. Later, still in the presence of carbachol, action potentials show a further increase in the negative after potential component and an eventual reduction in the initial spike component until

this was lost leaving a slow wave. Slow waves occurred sporadically at this lower temperature. At higher temperatures, repeated spikes took off from the negative after potential of the initial action potential.

Effects of reducing the external sodium concentration on the response to carbachol

As the longitudinal muscle layer of the guinea-pig ileum is extremely thin, about 30–50 μ in thickness (Paton, 1964), changing the ionic composition of the bathing fluid must result in fairly rapid changes in the ionic

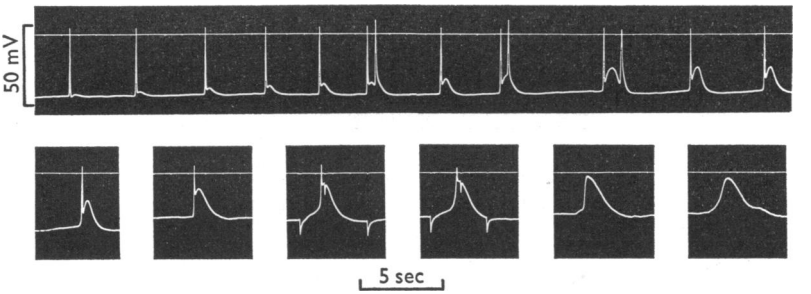

Fig. 7. Equivalence of the slow wave and negative after-potential. In the upper record carbachol was introduced to the bathing solution and as it took effect a negative after-potential appeared and then increased in size. The lower records were taken later at selected points of the trace to show how the spike component eventually declines leaving the slow wave. In the middle panels brief pulses were used to elicit electrotonic potentials to demonstrate that at the peak of the negative after-potential the membrane resistance was reduced (compare Fig. 5). Hypertonic solution at 27° C.

composition of the extracellular fluid, thus modifying the ionic gradients across the cell membrane. Changes in the external sodium concentration had profound effects on the membrane response to carbachol. Within 2 min of reducing the external sodium from 125 to 15 mM by replacing the sodium chloride with Tris chloride the response to carbachol was greatly attenuated (Fig. 8). No action potentials or slow waves were observed, but the membrane was capable of generating action potentials in response to depolarizing current (Fig. 8) although its ability to generate action potentials declined as the carbachol took effect (this was also true in normal solution). Spikes could be elicited by depolarizing current after more than 45 min in sodium-deficient solution. If the tissue was returned to solution of normal sodium composition the response to carbachol rapidly returned to normal (Fig. 8*A*). The possibility that the effects of solution 8 were due to a toxic or pharmacological blocking action of Tris was excluded by adding a larger amount of Tris to a solution contain-

ing the normal sodium concentration. In this solution the response to carbachol was similar to that obtained in normal solution except that slow waves were more frequent.

The abolition of carbachol-induced slow waves by sodium deficiency was not due to a reduction of the depolarization which carbachol normally produced as, when depolarizing pulses of several seconds duration were applied in sodium-deficient solution and in the presence of carbachol, slow waves were still not produced. However, action potentials were evoked by this procedure, although the ability of outward current to evoke spikes declined with increasing time in contact with carbachol.

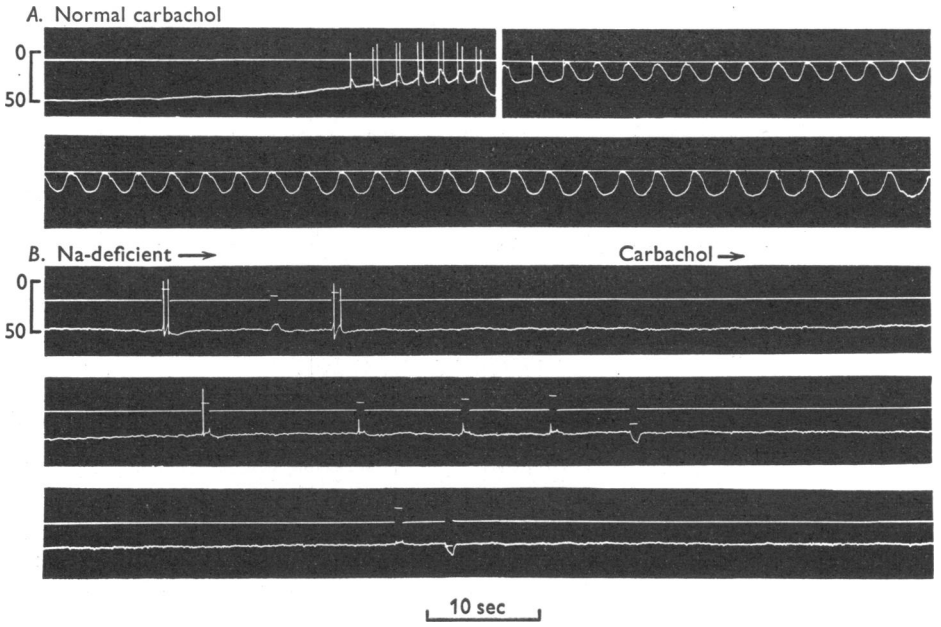

Fig. 8. Effects of sodium-deficient solution on the response to carbachol (5×10^{-7} g/ml.). *B* was obtained 6 min after changing to sodium-deficient solution. Carbachol does not produce either slow waves or spikes but depolarizing current pulses evoke action potentials, at least until the effects of carbachol are well advanced. After returning to normal solution, carbachol elicited both spikes and slow waves (*A*). The gap in the record represents a period of about 30 sec, otherwise the records under *A* and under *B* are continuous. Hypertonic solution 35° C.

DISCUSSION

Transmitter substances very likely produce their effects on excitable membranes by opening additional ion channels. The resistance of these channels to the passage of ions in most situations is unaffected by altera-

tions in the level of the membrane potential (see review by Ginsborg, 1967). It is probable that acetylcholine has this type of action on smooth muscle. The increment in voltage-independent conductance produced by acetylcholine would be expected to move the membrane potential towards the equilibrium potential for acetylcholine, and this would explain the reduction in membrane potential which acetylcholine produces. The additional conductance would also be expected to modify the expression of those voltage and time-dependent ion channels which are presumably responsible for the action potential (e.g. Fatt & Katz, 1951; Maeno, 1966). In smooth muscle, this additional conductance probably accounts for the reduced size and rate of rise of the spike, and the increase in spike frequency and in spike duration which are observed whenever acetylcholine produces any substantial depolarization.

However, the experiments described on this smooth muscle clearly indicate that acetylcholine or carbachol must do more than simply open an additional, voltage-independent, conductance in the membrane. The results in hypertonic solution show that the spike mechanism is a voltage-dependent one which operates whenever the membrane potential is reduced sufficiently below its resting level, whether the reduction in potential is brought about by passing current, by increasing the external potassium concentration, or by carbachol. This was not the case with slow waves, however, as these occurred in hypertonic solution only in the presence of a muscarinic stimulant, such as acetylcholine or carbachol and not otherwise (Fig. 4).

It might be argued that slow waves represent the operation of the voltage-dependent mechanism responsible for the spike, modified by the additional conductance introduced by carbachol. The experiment illustrated in Fig. 8 shows that this cannot be true: here spikes exist side by side with slow waves (or negative after potentials), and even take off from the peaks of slow waves, yet the forms of the spikes show only minor differences. Further support for the view that spikes and slow waves represent the operation of different mechanisms was obtained from the effects of sodium-deficient solution. This produced a rapid and reversible block of slow waves, but not of the spikes. Thus, in hypertonic solution, carbachol or acetylcholine 'switch on' the slow wave mechanism, and this mechanism can operate independently of the processes responsible for the spike.

Several experiments give an indication of the mechanism which might be involved in the production of the slow wave. In hypertonic solution it was shown that at the peak of the slow wave the membrane conductance was reduced, suggesting that a permeability change may be involved. The slow wave mechanism, although 'switched on' by acetylcholine or

carbachol, is nevertheless voltage dependent. Slow waves induced by carbachol were always abolished by hyperpolarizing current, and their rate of rise was a function of the level of the membrane potential from which they arose. They were abolished if concentrations of carbachol were used which depolarized the membrane to a steady level of about -15 mV, but reappeared if the membrane was electrically repolarized at this time. They were also abolished if the membrane was sufficiently electrically depolarized. These properties are very suggestive of the operation of a voltage-dependent ion channel. The effects of sodium deficiency indicate that the main ion carried by this channel may be sodium.

The action of acetylcholine or carbachol in isotonic solution was consistent with the above hypothesis. In isotonic solution, slow potentials occur spontaneously, and these carry bursts of action potentials. Acetylcholine increased the size of these slow potentials, so that the amplitude of the spikes was reduced. Thus slow potentials were converted into slow waves, indicating that the underlying mechanism of these two phenomena may be the same. Both in isotonic and in hypertonic solution the action of acetylcholine or carbachol is probably fundamentally similar, and it is suggested to be that of increasing the inward sodium current carried by a slow, voltage-dependent, ion channel. The suggested action of acetylcholine on the behaviour of a voltage-dependent channel is similar in principle to the action of adrenaline on cardiac muscle (Hauswirth, Noble & Tsien, 1968; Vassort, Rougier, Garnier, Sauviat, Coraboeuf & Gargouïl, 1969).

Hypertonic solution abolished the slow potentials (and also spikes, but the latter were evoked by depolarizing the membrane) and carbachol reversed this effect. It may be that hypertonic solution and carbachol have antagonistic effects on the current carried by the postulated slow channel. Thus the activity evoked by carbachol in hypertonic solution was very similar to the spontaneous activity seen in isotonic solution (cf. Figs. 1*a* and 3).

Spikes were unaffected by sodium deficiency. Hence, the presumed fast channel which gives rise to them can probably carry a significant amount of calcium current. Bülbring & Tomita (1970) reached similar conclusions for the taenia coli and ureter. In the latter tissue, the action potential shows a plateau component from which repetitive spikes are normally discharged (Kuriyama *et al.* 1967*a*). This plateau is also sodium sensitive (Bülbring & Tomita, 1970; Kuriyama & Tomita, 1970). It is possible that two regenerative channels are present in most smooth muscle cell membranes, one admitting mainly calcium and the other mainly sodium. The slow potential changes and the differences in the configuration of the action potential in various smooth muscles may simply reflect differences in the

relative time courses and current carrying capacities of these two regenerative channels.

Thus the action of muscarinic stimulants on the membrane of this smooth muscle may involve at least two distinct modes of action. One effect is an increase in the ionic current through voltage-independent channels, which reduces the membrane potential slightly, moving it towards the equilibrium potential for acetylcholine. The other effect may be to alter the relationship between a time and voltage-dependent current through a slow channel and the membrane potential (without apparently affecting the current responsible for the spikes) so that periodic oscillations of the membrane potential (slow waves) are produced.

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